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A420
R31
2/15

February 1956

ARS-33-15

United States Department of Agriculture
Agricultural Research Service



USE OF BRINE SHRIMP FOR THE DETECTION
OF INSECTICIDE RESIDUES

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Bioassay methods for the detection of insecticide residues have a strong appeal because of the broad range of compounds to which they can be applied and because of their high sensitivity. Most of the known bioassay methods require the rearing of test organisms in quantity, usually a time-consuming and more or less expensive procedure. Also many difficulties are often encountered in rearing these organisms in the laboratory.

In preliminary tests at Beltsville, Md., brine shrimp (Artemia salina) showed promise as a test organism for the detection of insecticide residues. Artemia salina is a salt-water crustacean about 12 mm. long. It is practically world-wide in distribution, and has been the subject of physiological studies by zoologists for many years. More popularly this organism has found wide use as a food for tropical fish, and the eggs are obtainable at most tropical-fish stores. The dry eggs can be kept on the laboratory shelf for several years without losing their viability. If they are placed in a suitable hatching solution, the nauplii, or young shrimps, will emerge within 30 hours. The necessity of maintaining cultures is thus eliminated and yet the organism is available the year around. These initial experiments disclosed the following pertinent facts concerning the use of brine shrimps as test organisms for the bioassay of toxic materials: All age groups from 24-hour old nauplii to adults are sensitive to the presence of insecticides in micro amounts. Brine shrimps are constantly in rapid motion, so that any cessation or reduction in activity can be used as a criterion of toxicity. They will tolerate a wide range of salt concentrations and will survive in distilled water long enough to obtain valid tests. Therefore, suspensions of toxic materials can be prepared and tested in either water or salt solutions. The shrimps will tolerate large amounts of organic solvents; for example, adult shrimps will live for more than 48 hours in a 1:100 solution of acetone in distilled water.

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Rearing of Brine Shrimps

Brine shrimps may be reared in the laboratory with a minimum of effort and expense. A salt solution of the following composition is prepared:

	<u>Grams</u>
Sodium chloride	60
Calcium sulfate	3.4
Magnesium sulfate	6.4
Magnesium chloride	17
Potassium chloride	1.6
Sodium bromide	0.2
Distilled water	1000 ml.

The pH of this solution is adjusted to 9.5 with sodium hydroxide. For hatching and rearing purposes, this salt solution is diluted with distilled water to half-strength. For bioassay testing it is used full-strength.

The salt solution is placed in a glass tray approximately 16 by 12 inches and 2 to 3 inches deep, and a wooden divider is set about 5 inches from one end. The eggs are then sprinkled on the surface of the solution on the narrow side of the divider. When the eggs hatch, the nauplii swim under the divider and can be drawn to the clear end of the tray by positive phototaxis. Here they are readily collected, free of eggs, by siphoning or suction.

The eggs in the solution are incubated at 30° C. Hatching begins after 18 hours and is complete after 30 hours. At any time during this period the nauplii may be transferred to other containers.

The shrimps are reared from nauplii to adults in approximately 3 weeks on yeast, which is an excellent and convenient food. A moderately heavy suspension of yeast is made in water, and from 1 to 10 drops are floated on the solution, depending upon the number of shrimps and the need for food as evidenced by the cloudiness of the solution. An excess of food is detrimental. The optimum temperature is about 30° C., but they will live for a time at temperatures as low as 10° and as high as 37°.

Extraction of Insecticide Residues

The method of extracting an insecticide residue from a sample depends upon the nature of the sample and the degree of purification desired. The methods of extraction and purification as outlined by Hoskins^{1/} are satisfactory. A random sample of not less than

^{1/} Hoskins, W. M. Purification of plant material and separation of insecticides for bioassay. Panel presentation at the Joint Raw Products Laboratory Session, National Canners' Association Convention at Chicago, Ill., on February 21, 1955.

1000 grams is finely macerated in a blender and then extracted with 1000 ml. of solvent by shaking. With wet samples, anhydrous sodium sulfate is added to tie up the water so that a single liquid phase is formed. Gross material can be eliminated by filtration or the use of water dilution and a separatory funnel. The use of equivalent portions of sample and solvent means that for analysis each 1 ml. of solvent will represent 1 gram of sample. The recovered extract is evaporated to one one-hundredth its volume in a stream of warm air and then brought back up to full volume again with the suspending liquid, either salt solution or distilled water.

Most of the experimental work has been done on residues from honey bees killed by insecticides. The toxicant is extracted from 100 bees, which is a convenient and adequate sample, by thoroughly macerating them with 10 grams of anhydrous sodium sulfate and 100 ml. of acetone. After filtering through a No. 2 filter paper, the acetone solution is placed in a small beaker and evaporated to 1 ml. in a stream of warm air. To this material 10 ml. of full-strength salt solution is added. The suspension that forms is again filtered and the volume brought up to 100 ml. with full-strength salt solution. With these proportions 1 ml. of suspension will contain the toxicant from one bee and any aliquot volume used for analysis will have the same relationship.

Bioassay

Two milliliters of the suspension is placed in a 10- by 75-mm. test tube, together with five 3-weeks-old adult brine shrimps that have been washed twice in salt solution to remove any extraneous organic matter that may interfere with the test. Five replicates are used in each test. Check suspensions are also tested at the same time. The shrimps move freely and rapidly up and down the column of liquid. However, when toxic material is present, it affects their swimming organs and, owing to the density of the salt solution, they float to the top of the column of liquid. This is the criterion used for making readings. The time when the shrimps are affected indicates the amount of toxic material present. Time-flotation graphs can be prepared from data obtained by exposing shrimps to known amounts of pure toxicants or of toxicants recovered from the same kind of sample. By comparison with the data obtained from the test sample the amount of toxicant present can be read directly. An alternative method of computing the amount of toxicant in the sample, if the approximate level of residue is known, is to test a series of standards at the same time and make direct comparisons.

Suspensions prepared with distilled water are tested in the same way.

Shrimps 72 hours old can be used in the same way, but the adult was made the final choice of test organism because of the ease of handling and readings are more readily made, with little need for interpretation.

Bioassays can also be made with shrimps 24 hours old on porcelain or glass spot plates. From 9 to 12 tests can be run on one plate. The plate is easily handled and can be placed beneath a microscope for direct counting. A specific number of drops of suspension and approximately 50 nauplii are placed in each spot. The plates are then placed in a desiccator that has been transformed into a moist chamber by placing a roll of cotton in the base. In this moist chamber the young shrimps will live at least 5 days. However, symptoms of toxicity will be demonstrated within 1 hour. The chief difficulty encountered with this method is that the rapid movement of these small shrimps makes readings difficult and necessitates a certain reliance upon the interpretive ability of the reader.

Tests with Various Insecticides

Experiments were made with lindane, methoxychlor, chlordane, DDT, toxaphene, endrin, and Bayer 17147 as test insecticides. One-percent solutions in acetone were prepared from which suspensions of different concentrations were made in full-strength salt solution. Washed brine shrimps were placed in these suspensions. Toxic action was determined by loss of swimming ability. Results obtained are shown in table 1.

Table 1.--Hours required for flotation of shrimp in suspensions of insecticides in full-strength salt solution.

Insecticides	1 p.p.m.	0.1 p.p.m.	0.01 p.p.m.
Methoxychlor	1	1	1
DDT	1	1	2
Lindane	1	2	2
Chlordane	2	2 $\frac{1}{2}$	3
Toxaphene	1	2	18
Endrin	1	2	18
Bayer 17147	3	6	18

A double-strength salt solution containing acetone (1:100) and a full-strength salt solution alone required more than 48 hours for shrimp flotation.

The viability of different lots of shrimp eggs varies, and there is some evidence that lots of adults differ in sensitivity when tested against insecticides. However, by standardizing each lot of shrimp before testing and by use of adequate controls, these differences can be reduced.

